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THE EFFECTS OF PRALIDOXIME, ATROPINE, AND PYRIDOSTIGMINE ON THERMOREGULATION AND WORK TOLERANCE IN THE PATAS MONKEY

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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A continuous flow indirect calorimetry system was used to continuously measure metabolic rates in patas monkeys at rest or during exercise when exposed to ambient temperatures of 25 °or 35 °C. The exercise system was also used to evaluate the effects of pralidoxime, atropine, and/or pyridostigmine on the exercise tolerance time in primates. Rectal temperature and heart rate were continuously monitored by a telemetry system while total evaporative water loss was estimated from weight differences before and after exercise. Resting metabolic rates measured at 35 °C were significantly higher than at 25 °C and averaged 73.3 and 49.1 W/m^2 respectively. No significant difference was observed in the exercising metabolic rates of the patas monkey measured at 25 °C which averaged 126.3 and that at 35 °C which averaged 123.4 W/m^2. No significant drug effects on metabolic rate or respiratory quotient were observed. Pyridostigmine treatment was associated with an increase in exercise time, a lower rectal temperature, heart rate, and a 40% increase in water loss. Atropine treatment produced a decrease in exercise time of 61 min and water loss, and an increase in heart rate. Pralidoximine treatment alone or in combination with atropine had no significant effect on exercise time or thermoregulation. Pyridostigmine treatment in combination with atropine resulted in a significant increase in exercise time and a 47% increase in water loss when compared to atropine treatment alone. Therefore, moderate inhibition of serum cholinesterase activity may partially reverse the debilitating effects of atropine on physical performance and thermoregulation during exercise in the heat.

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THE EFFECTS OF PRALIDOXIME, ATROPINE, AND PYRIDOSTIGMINE ON THERMOREGULATION AND WORK TOLERANCE IN THE PATAS MONKEY

INTRODUCTION

Pralidoxime chloride is currently used as an antidote to organophosphate (OP) poisoning. The action of pralidoxime centers around the reactivation of bound acetylcholinesterase (AChE) which is then available for the hydrolysis of acetylcholine (ACh). This therapeutic approach facilitates the normal function of synapses following OP poisoning (11). The effect of pralidoxime on thermoregulatory sweating remains unclear since previous studies have reported either no change or a decrease in sweating in exercising subjects (4,12,15).

Atropine, a common antidote for OP (11), suppresses thermoregulatory sweating and evaporative heat loss through its anticholinergic activity with increased heat storage and decreased heat and exercise tolerance (6,12).

Pyridostigmine is used as a prophylactic against anticholinesterase (anti-ChE) poison by reversibly inhibiting cholinesterase (ChE). Depending on the degree of enzyme inhibition and mode of administration, this drug affects exercise performance and heat tolerance in a negative (8) or positive way (9).

In a previous study, the physiological effects of a single intramuscular (i.m.) injection of atropine or oral pyridostigmine treatment on thermoregulatory capacity and exercise tolerance time of patas monkeys exposed to ambient temperatures (Ta) of 25 °C and 35 °C were investigated (7). Atropine effects were more pronounced at Ta 35 °C as indicated by a significant reduction in evaporative water loss (EWL) (43%) which was associated with an average exercise time that was 56% less than the control no-drug treatment condition. The final heart rate (HR) and rectal temperature (Tre) response in these atropine experiments were significantly elevated above control values. On the other hand, pyridostigmine significantly increased EWL (61%) which was associated with an average exercise time of 60 min longer than the control value. The final HR and Tre responses were not significantly affected by the pyridostigmine treatment.

In our study we were interested in assessing the effects of pralidoxime chloride, atropine, and/or pyridostigmine alone and also the combined effects of pralidoxime chloride, atropine, and/or pyridostigmine on the thermoregulatory effectors and exercise tolerance of patas monkeys exposed to 25 °C and 35 °C environments.

METHODS AND MATERIALS

Design of Indirect Calorimetry System

A continuous flow indirect calorimetry system was developed consisting of a resting metabolic cage or an exercising cage in which the monkey resided (7). Fresh air

was pulled through the cage by a fan and the airflow out was measured by a turbine flow meter. Airstream temperature and water vapor pressure were measured before a small aliquot of gas was sampled for oxygen (O_2) and carbon dioxide (CO_2) content. Calculation of metabolic parameters from the measurement of O_2, CO_2, P_{H2O}, P_B, flow, and temperature was done using the algorithms of Brown (2), which account for the storage (integration) of gas volumes in the resident cage. This system was used to continuously measure metabolic rates in patas monkeys at rest following equilibration to Ta of 25 °C or 35 °C, and during exercise following pralidoxime, atropine, and/or pyridostigmine treatment at 35 °C Ta.

Design of Primate Exercise Device

The apparatus used to evaluate the exercise tolerance of patas monkeys was an ergometer specifically constructed to exercise nonhuman primates. The wheel consists of two 122-cm (48 in.) diameter Lucite rings and 120 aluminum bars which form a circular activity wheel which rotates freely on 4 bearings (5). A copper ring, affixed to the outside of each Lucite ring, was connected to alternate aluminum bars to provide electrical stimulation for conditioning the animal. The activity wheel included: (1) a magnetic tachometer pickup to quantitate speed; (2) an automatic control panel interconnected with the tachometer to allow the setting of upper and lower speed limits which the animal had to maintain in order to avoid a sequence of visual or electrical stimuli; (3) a microswitch at the bottom of the control panel which counted each revolution of the wheel and calculated distance traveled; and (4) a brake to stabilize the wheel during the rest periods as well as to prevent the animal from operating the wheel at higher than predetermined rates.

Exercise Program Operation

Six patas monkeys were trained to operate the ergometer until they learned to run at a minimum rate of 2 miles/hour (mph) for 60 min (7,13).

At the end of a 20-week training period, each animal was capable of completing at least 1 h of exercise while control values of exercising HR and Tre were measured every 15 min by a noninvasive telemetry system. The system consisted of an AMF Quantum XL transmitter for measuring HR and a mini-mitter rectal probe transmitter for measuring Tre. Heart rate was recorded by an AMF Quantum digital watch receiver in beats per minute (bpm) while Tre was recorded by a President AX52 FM receiver and converted to degrees centigrade using calibration curves validated in the laboratory. Total distance covered in miles, average speed in miles per hour, and total exercise time were also recorded. Water loss was estimated from weight difference measured before and after the exercise test.

The criteria used to establish the exercise tolerance of the animals and to terminate the standard exercise test were any one of the following: (1) HR that approach maximal HR for this species, about 300 bpm; (2) a Tre higher than 40 °C to protect the animals from heat injury; or (3) going 3 times underspeed (2 mph) in each of 2 consecutive 15-min periods.

The exercise tolerance tests were repeated following pralidoxime, atropine and/or pyridostigmine treatment alone or in combination.

Treatment Protocol

Two days after each animal completed a control exercise test at 25 °C, another exercise test was carried out at 35 °C. Three days later the same procedure was repeated following an 8.5 mg/kg single i.m. pralidoxime chloride injection or a 0.03 mg/kg single i.m. atropine injection. Three days later the exercise test was repeated following 0.03 mg/kg atropine plus 8.5 mg/kg pralidoxime i.m. administrations.

In another series of experiments each animal was treated orally with 2 separate doses of solid 0.4 mg/kg pyridostigmine: 1 in the morning and 1 in the afternoon. The afternoon of the next day, the animal repeated the exercise test after receiving 2 additional oral doses of 0.4 mg/kg pyridostigmine: 1 in the morning and one 1 h before exercise plus an i.m. injection of 0.03 mg/kg atropine given immediately before exercise. Blood samples, from the saphenous vein, were taken before and after pyridostigmine treatment and assayed for serum ChE levels (14).

Treatment of Data

The results are presented as means and standard deviation. They were analyzed by a 2-way analysis of variance (ANOVA) with repeated measures with 1 fixed factor (drug condition) and 1 random factor (animal). Tukey's test of critical differences was also used where appropriate. All significant differences are reported at $p < .05$, unless otherwise noted.

RESULTS

Figure 1 illustrates the respiratory quotient during exercise associated with each drug treatment condition. Atropine treatment was associated with the highest respiratory quotient and averaged 0.87 ± .01 while atropine treatment in combination with pyridostigmine was associated with the lowest respiratory quotient and averaged 0.78 ± .02. None of the drug treatment respiratory quotients, however, were significantly different from no-treatment control. In addition, no significant differences in respiratory quotients were observed between experiments performed at a Ta of 25 °C when compared to those at a Ta of 35 °C.

The effects of Ta on the resting metabolic rate of the patas monkey are illustrated in Figure 2. Resting metabolic rate at 25 °C averaged 49.1 ± 2.0 W/m^2 and was significantly increased to 73.1 ± 10 W/m^2 following thermal equilibration at a Ta of 35 °C. Resting Tre after equilibration at Ta of 25 °C averaged 38.1 ± 0.3 °C and was not significantly different from the Tre measured following equilibration to Ta of 35 °C, which averaged 38.3 ± 0.2 °C.

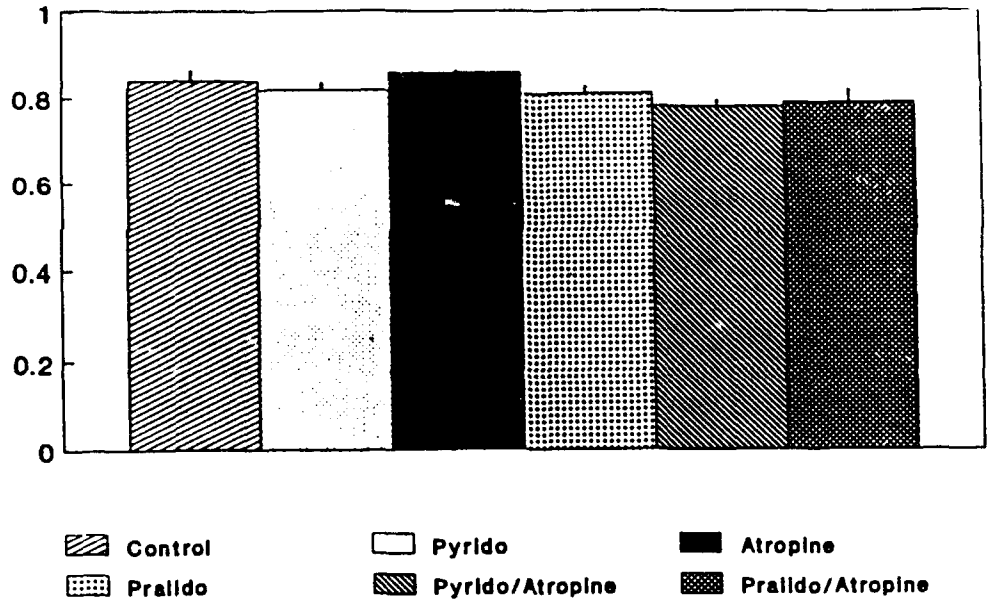

Figure 1. Respiratory quotient vs. drug condition at ambient temperature of 35 °C.

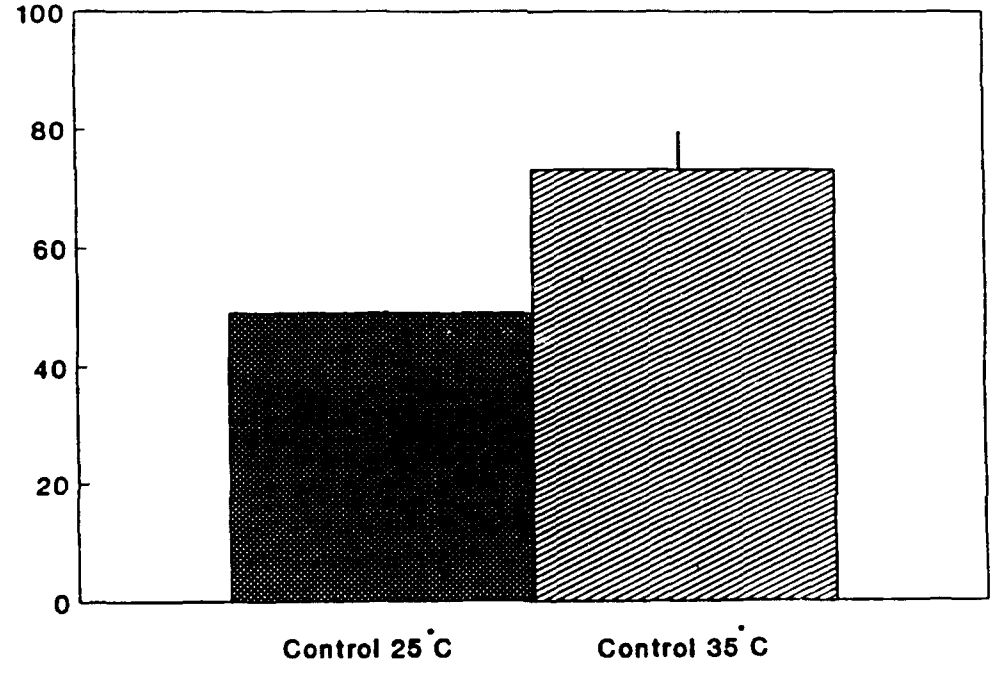

Figure 2. Resting metabolic rate (W/m^2) at ambient temperatures of 25 °C and 35° C.

As illustrated in Figure 3, no significant difference was observed in the exercising metabolic rate of the patas monkey measured at a Ta of 25 °C which averaged 126.3 ± 12 and the rate measured in exercising monkeys at 35 °C which averaged 123.4 ± 6.0. Table 1 shows that the Tre at the end of exercise at a Ta of 35 °C was 39.9 ± 0.1 °C and was significantly higher than the Tre at the end of the exercise at a Ta of 25 °C which averaged 39.6 ± 0.2 °C. Exercise at a Ta of 35 °C was also associated with a significant 92% increase in total water loss and a significant increase in final HR compared to 25 °C results. There was also a significant decrease in exercise time which averaged 31 min less than the exercise tolerance time recorded at an ambient temperature of 25 °C.

The exercise metabolic rates associated with each drug treatment are illustrated in Figure 4. The metabolic rate was highest following atropine treatment and averaged 128 ± 6.0 W/m^2 and lowest following pralidoxime treatment and averaged 110 ± 7.0 W/m^2. None of the metabolic rates associated with any drug treatment differed significantly from the no-treatment 35 °C control.

The effects of the various drug treatments on exercise tolerance and other physiological measurements of the study are summarized in Table 1. We can see that pyridostigmine treatment in this study was associated with a significant increase in exercise time which averaged 28 min longer than the no-treatment control. Pyridostigmine was also associated with a significant 44% increase in total EWL. Atropine treatment, on the other hand, was associated with a significant decrease in exercise time which averaged 61 min less than the 35 °C control. Atropine also resulted in a significant increase in exercising HR and a significant 40% decrease in EWL. As seen in Table 1, pralidoxime treatment was associated with a 20% decrease in EWL which did not reach statistical significance. Pralidoxime treatment in combination with atropine produced no meaningful synergistic effects that were different from atropine treatment alone. Pyridostigmine treatment in combination with atropine tended to attenuate the effects of atropine alone relative to exercise time while also increasing (47%) the EWL as compared to atropine treatment alone. As illustrated in Figure 5, pyridostigmine treatment was also associated with a significant 21% decrease in serum ChE activity.

DISCUSSION

The results of these studies indicate that the effects of pralidoxime chloride, atropine, and pyridostigmine in the patas monkey are qualitatively similar to those reported for humans. It has been reported that an i.m. injection of 2 mg of atropine, a dose analogous to the one administered in this study, reduced sweating capacity in humans between 45 and 55% and is similar to the 40% decrease observed in this study (6,7,12). The suppressed sweating caused by atropine resulted in increased heat storage and a significant decrease in exercise time. All atropine runs had to be terminated because the monkeys failed to reach thermal equilibrium. The experiments were terminated when Tre reached between approximately 40.0 - 40.5 °C to prevent heat injury. Atropine was also observed to increase HR since this dose of atropine has been shown to result in nearly maximal vagal inhibition (3). In this study, mean exercise HR increased by 21 bpm and mean Tre increased by 0.3 °C in atropine-treated monkeys exercising in the heat. When pralidoxime was administered in combination with atropine, no synergistic effects were observed. In addition, none of the physiological parameters measured in this study were significantly affected when the monkeys were treated with pralidoxime alone.

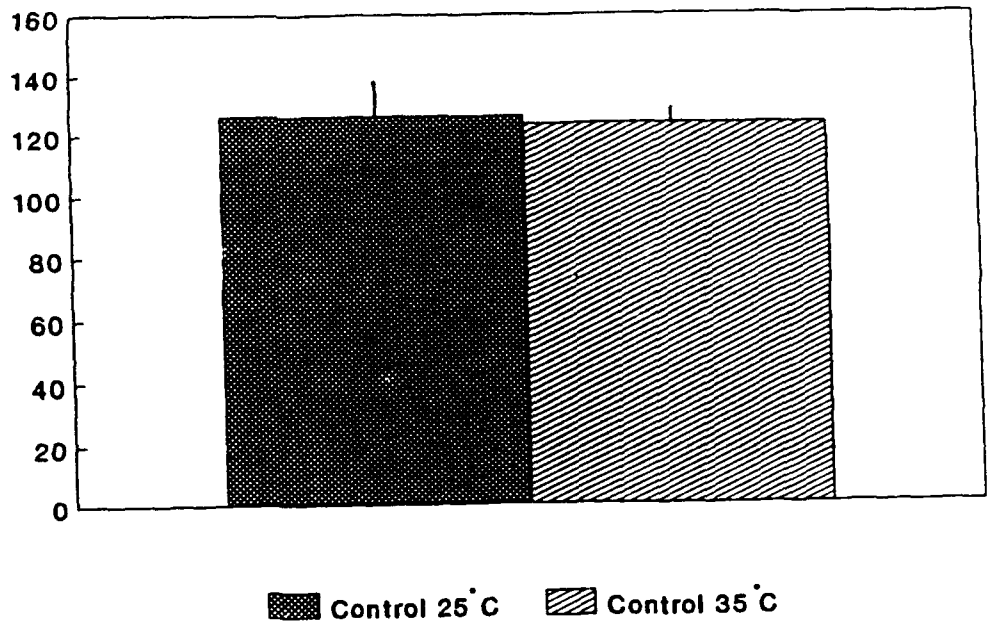

Figure 3. Exercising metabolic rate (W/m^2) at ambient temperatures of 25 °C and 35 °C.

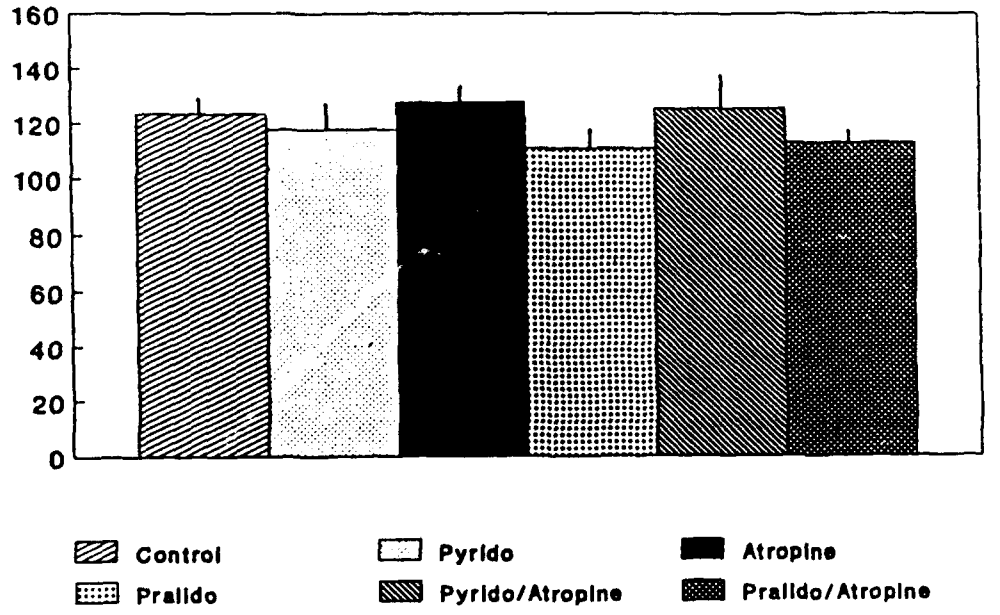

Figure 4. Exercising metabolic rate (W/m^2) vs. drug condition at ambient temperature of 35 °C.

TABLE 1. SUMMARY OF RESULTS AT END OF EXERCISE

	Time (min)	Speed (mph)	Distance (mi)	T_{re} (^{o}C)	HR (bpm)	E_{tot} (g/min)
Control 25 °C	175 ± 34	1.6 ±.05	4.7 ±1.0	39.6 ±0.2	212 ±7.0	1.3 ±1.7
Control 35 °C	144[a] ± 40	1.5 ±.01	3.6[a] ±0.7	39.9[a] ± 0.1	219[a] ±9.0	2.5[a] ±2.0
Pyrido 35 °C	172[b] ± 25	1.6 ±0.1	4.6 ±1.0	39.2 ± 0.3	214 ±10.0	3.6[b] ±2.0
Atropine 35 °C	83[b] ±13	1.8[b] ±0.2	2.4[b] ±0.7	40.2 ±0.2	240[b] ±12.0	1.5[b] ±2.0
Pralido 35 °C	140 ± 15	1.5 ±.02	3.5 ±0.8	39.8 ± 0.3	220 ±11.0	2.0 ±2.0
Pyrido/Atropine (35 °C)	100[b] ± 15	1.7 ±0.2	2.8 ±0.8	39.7 ± 0.3	237[b] ±10.0	2.2 ±2.0
Pralido/Atropine (35 °C)	88[b] ±20	1.7 ±0.1	2.5[b] ±0.5	40.4 ± 0.2	242[b] ±11	1.5[b] ±0.8

[a] $p < .05$ Compared to Control 25 °C
[b] $p < .05$ Compared to Control 35 °C
(N = 6 values are $\bar{X}$ ± S.D.)

Francesconi et al. (8,9) reported in rats that pyridostigmine treatment which produced ChE inhibition of 23% and 39% significantly improved exercise performance and thermoregulatory capacity during exercise in the heat. This study supports the findings of Francesconi et al. in a primate animal model. We found that the oral administration of 0.4 mg/kg pyridostigmine was associated with moderate ChE inhibition which averaged 21%. In our study, pyridostigmine treatment was associated with a significant 44% increase in total EWL, a lower Tre and was associated with a 28 min longer exercise time than the no-drug treatment control. When pyridostigmine was administered in combination with atropine, there was a 17-min increase in average exercise time compared to atropine alone. The increased exercise time was associated with a 47% increase in total EWL and 0.5 decrease in Tre as compared to atropine treatment alone. In summary, this study in a primate model supports the notion that moderate ChE inhibition can provide some protection against the debilitating effects of atropine on physical performance and thermoregulation during exercise in the heat. Finally, a pilot set of experiments was run on 3 monkeys in an attempt to study the effects of 1 h of exercise immediately before drug treatment (atropine or pralidoxime) followed by an exercise tolerance test. We observed that 1 h of exercise at a Ta of 35 °C was consistently associated with Tre that exceeded 39.6 °C. Furthermore, the stress of the drug injection and the start of the exercise tolerance run produced a further rapid rise in Tre that exceeded 40.5 °C within 5 min after drug treatment. The experiments were terminated at this point to protect the animals from heat illness and before any steady-state data could be obtained.

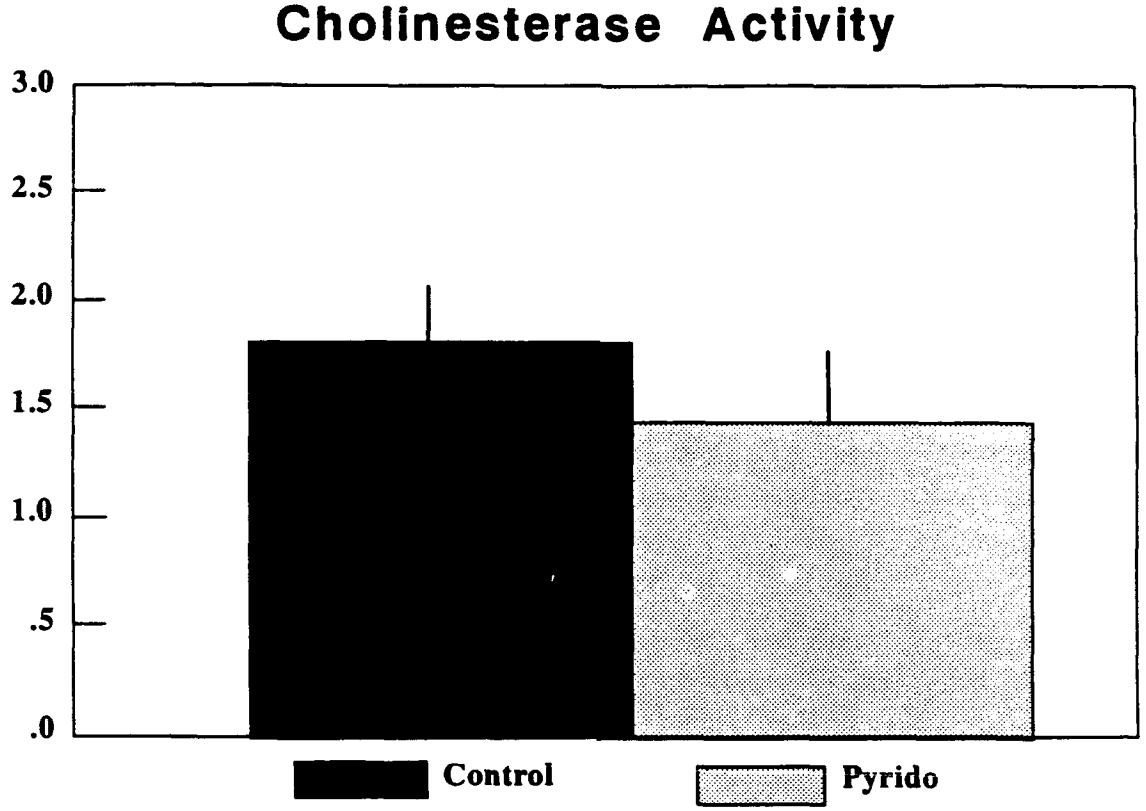

Figure 5. Serum ChE activity before and after pyridostigmine treatment (I.U.).

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APPENDIX

TABLE A-1. RESPIRATORY QUOTIENT VS. DRUG CONDITION

Drug (35 °C)	Respiratory Quotient		
Control	.84	+/-	.03
Pyridostigmine	.82	+/-	.02
Atropine	.86	+/-	.01
Pralidoxime	.81	+/-	.02
Pyridostigmine/Atropine	.78	+/-	.02
Pralidoxime/Atropine	.79	+/-	.03

TABLE A-2. RESTING METABOLIC RATE (W/m^2)

Drug	Resting Metabolic Rate (W/m^2)		
Control (25 °C)	49.1	+/-	2.0
Control (35 °C)	73.1	+/-	12.9

TABLE A-3. EXERCISING METABOLIC RATE (W/m^2)

Drug	Exercising Metabolic Rate (W/m^2)		
Control (25 °C)	126.3	+/-	12.5
Control (35 °C)	123.4	+/-	5.5

TABLE A-4. METABOLIC RATE (W/m^2) VS. DRUG CONDITION

Drug (35 °C)	Metabolic Rate (W/m^2)		
Control	123.4	+/-	5.5
Pyridostigmine	117.8	+/-	9.6
Atropine	128.0	+/-	6.0
Pralidoxime	110.8	+/-	7.3
Pyridostigmine/Atropine	125.6	+/-	12.1
Pralidoxime/Atropine	113.0	+/-	4.9

TABLE A-5. CHOLINESTERASE ACTIVITY

Drug	Rate (moles/ml/min)		
Control	17.7	+/-	3.0
Pyridostigmine	14.1	+/-	2.5